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Monoclonal Antibodies to Rat Brain Acetylcholinesterase: Comparative Affinity for Soluble and Membrane-Associated Enzyme and for Enzyme from Different Vertebrate Species

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Abstract: Seven unique monoclonal antibodies were generated to rat brain acetylcholinesterase. Upon density gradient ultracentrifugation, immunoglobulin complexes with the monomeric enzyme appeared as single peaks of acetylcholinesterase activity with a sedimentation coefficient approximately 3S greater than that of the free enzyme. This behavior is consistent with the assumption of one binding site per enzyme molecule. Apparent dissociation constants of these antibodies for rat brain acetylcholinesterase calculated on the basis of this assumption ranged from about 10 n*M* to more than 1,000 n*M*. Some of the antibodies were less able to bind the membrane-associated enzyme that required detergent for solubilization than the naturally soluble acetylcholinesterase of detergent-free brain extracts. Species cross-reactivity was investigated with crude brain extracts from mammals (human, mouse, rabbit, guinea pig, cow, and cat) and from other vertebrates (chicken, frog, and electric eel). Three antibodies bound rat acetylcholinesterase exclusively; one had nearly the same affinity for all mammalian acetylcholinesterases investigated; the remaining three showed irregular binding patterns. None of the antibodies recognized frog and electric eel enzyme. Pooled antibody was found to be suitable for specific immunofluorescence staining of large neurons in the ventral horn of the rat spinal cord and smaller cells in the caudate nucleus. Other potential applications of these antibodies are discussed. **Key Words:** Acetylcholinesterase—Mammalian brain—Monoclonal antibodies—Immunohistofluorescence. **Rakonczay Z. and Brimijoin S.** Monoclonal antibodies to rat brain acetylcholinesterase: Comparative affinity for soluble and membrane-associated enzyme and for enzyme from different vertebrate species. *J. Neurochem.* **46,** 280–287 (1986).

Immunochemical methods can detect subtle structural differences in the apparently homogeneous acetylcholinesterases (AChE; EC 3.1.1.7) isolated from different sources. Accordingly, these methods are becoming powerful tools for analyzing the structure and localization of AChE, as well as the function of this enzyme in cholinergic neurotransmission.

Rodent brain AChE is particularly suitable for immunochemical study, since its physiology and distribution are of great interest and much is already known about its molecular heterogeneity and biochemical properties (Rakonczay et al., 1981*a,b*). However, there are only a few reports of specific antibodies to the AChE of rat or mouse brain (Adamson, 1977; Greenberg et al., 1977; Zanetta et al., 1981; Marsh et al., 1984). Furthermore, all of the reported antibodies are polyclonal, a fact that limits their usefulness for detailed investigation.

Recently, monoclonal antibodies have been produced to the AChE of human red blood cells (Fambrough et al., 1982; Brimijoin et al., 1983) and rabbit brain (Mintz and Brimijoin, 1985). These antibodies have permitted some insights into the organization of the mammalian enzyme. Unfortunately, they have little affinity for rat brain AChE.

Perhaps because of immunochemical similarities among the AChEs of rodents (Marsh et al., 1984),

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Abbreviations used: AChE, acetylcholine acetylhydrolase, EC 3.1.1.7; IgG, immunoglobulin G.

it has been difficult to produce murine monoclonal antibodies to rat brain AChE. However, by subjecting this enzyme to partial heat denaturation, we were able to generate high titers of circulating anti-AChE antibody in immunized mice. The goal of this study was to produce and characterize monoclonal antibodies to rat brain AChE. Here we describe the general properties, affinity, and species cross-reactivity of seven such antibodies.

MATERIALS AND METHODS

Rat, rabbit, and guinea pig brains (unstripped, mature) were purchased from Pel-Freez Biologicals (Rogers, AR, U.S.A.); cat brain was supplied by Dr. T. Yaksh (Dept. of Neurosurgery, Mayo Clinic); human brain was obtained at autopsy by Dr. H. Okazaki (Dept. of Pathology, Mayo Clinic). Inbred mice were supplied by Dr. C. David (Dept. of Immunology, Mayo Clinic). Complete Freund's adjuvant was purchased from DIFCO Laboratories (Detroit, MI, U.S.A.). Rabbit antiserum to mouse immunoglobulin was purchased from Miles Laboratories (Elkhart, IN, U.S.A.). The FO mouse myeloma cell line was a gift of Dr. G. Fathman (Dept. of Immunology, Stanford University). Pansorbin was obtained from Calbiochem-Behring (La Jolla, CA, U.S.A.). DEAE-Affi-Gel Blue was from BioRad Laboratories (Richmond, CA, U.S.A.). BW 284C51 [1,5-bis(allyldimethylammoniumphenyl)-pentane-3-one dibromide] was purchased from Burroughs Wellcome Co. (Research Triangle Park, NC, U.S.A.). Other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Enzyme assay and protein determination

The activity of AChE was measured spectrophotometrically (Ellman et al., 1961) or radiometrically (Johnson and Russell, 1975). BW 284C51 dibromide (10^{-5} *M*) and ethopropazine (10^{-4} *M*) were used, respectively, as specific inhibitors of AChE (to generate blank samples) and pseudocholinesterase (to eliminate interference in the assay). Enzyme activity was calculated in units of μmol substrate hydrolyzed per minute.

Protein was measured routinely by the dye-binding assay of Bradford (1976) or, if the sample contained high concentrations of Triton X-100, by the fluorescamine assay (Schwabe, 1973). Bovine serum albumin was the reference standard for both methods.

Preparation of brain extracts

Crude extracts for use as test antigen were made by homogenizing whole brains (fresh or frozen) in 10 volumes of buffer [12.5 m*M* sodium phosphate (pH 7.4), 0.4 *M* NaCl, with 0.5% (vol/vol) Triton X-100]. These extracts were centrifuged at 100,000 *g* for 1 h at 4°C and the supernatant fractions designated "total AChE." "Membrane-associated" and "soluble" AChE were prepared by extraction with or without detergent, as previously described (Rakonczay et al., 1981*b*).

Sucrose density gradient centrifugation

The AChE molecular forms were separated on linear 5–20% sucrose gradients (Rakonczay et al., 1981*b*). Samples (500 μl) were centrifuged for 15 h at 38,000 rpm at 4°C (SW-41 rotor, L8-55 ultracentrifuge; Beckman Instruments, Palo Alto, CA, U.S.A.). Catalase (11.3S) and bovine serum albumin (4.3S) were used as sedimentation markers.

Immunization and fusion

The AChE from adult rat brain was purified to homogeneity as described previously (Rakonczay et al., 1981*a*). On day 1, BALB/c mice were injected subcutaneously with 20 μg of highly purified rat brain AChE (partially heat-denatured at 52°C for 10 min) emulsified in complete Freund's adjuvant. On days 28 and 42, mice received booster immunizations with the same amount of enzyme, injected intraperitoneally, without adjuvant. On day 45 the spleens were removed and fused with FO myeloma cells. The hybridization was performed in the presence of polyethylene glycol by a modification (Fazekas de St. Groth and Scheidegger, 1980) of the procedure of Köhler and Milstein (1975), as fully described earlier (Brimijoin et al., 1983). Hybridomas were selected by culture in the presence of hypoxanthine, aminopterine, and thymidine. As cell densities approached confluency, the culture supernatants were tested for production of antibodies to rat brain AChE. Primary cultures secreting antibodies against the enzyme were subcloned by two successive limiting dilution steps (average of 1/3 cell per well) and were also visually inspected for the presence of single colonies.

Ascites tumors, antibody purification, and isotyping

Selected hybridoma clones were expanded by injection of 10^6 cells into the peritoneal cavities of female BALB/c mice primed 1 week earlier with Pristane (2,6,10,14,-tetramethylpentadecane; 0.3 ml, i.p.). The murine monoclonal antibodies were purified from the ascites fluid by chromatography on DEAE Affi-Gel Blue by the method of Bruck et al. (1982). Immunoglobulin isotypes were characterized with the aid of an enzyme-linked immunoassay kit (Zymed Laboratories, San Francisco, CA, U.S.A.).

Binding assay

A solid-phase immunoabsorbance assay was used to measure the binding of AChE (Brimijoin et al., 1983). Briefly, rabbit antimouse immunoglobulin and monoclonal antibody or normal mouse serum were adsorbed sequentially to Protein A-bearing Pansorbin to ensure quantitative precipitation of immune complexes. After several washings, the solid phase was resuspended and incubated with antigen for 1 h at 37°C. The extent of binding was determined after low-speed centrifugation by assaying the AChE activity in both supernatants and pellets.

Immunohistochemistry

Rats were perfused through the aorta with 100 ml of cold 0.9% NaCl followed by 200 ml of cold 0.1 *M* sodium phosphate (pH 7.4) containing 2% (wt/vol) paraformaldehyde (a concentration found optimal in preliminary work). Brain and spinal cord were then removed and immersed in the fixative for 2 h in vitro, followed by several rinses and an overnight incubation at 4°C in 0.1 *M* phosphate buffer with 5% sucrose as a cryoprotectant. Cross-sections of spinal cord and sagital sections of brain (12 μm) were cut on a cryostat, thawed onto gelatin-coated microscope slides, and processed by the indirect immunofluorescence technique of Coons (1958). The best re-

sults were obtained with pooled antibody [ZR 1-7, in equal parts, diluted 1:30 in 0.1 *M* sodium phosphate (pH 7.4) with 0.3% Triton X-100]. An equivalent dilution of normal mouse serum served as a control. Affinity-purified goat anti-mouse immunoglobulin G (IgG) conjugated with fluorescein isothiocyanate (Southern Biotechnology Associates, Birmingham, AL, U.S.A.) was the labeled second antibody. Tissue fluorescence was observed under epi-illumination in a Zeiss Axiomat microscope equipped with a silicon-intensified-target video camera (Dage-MTI, Michigan City, IN, U.S.A.) and Conrac video monitor. Photographs were taken directly from the monitor with a Polaroid CU-5 camera and type 552 film (0.5 s at f/11).

Statistical calculations

Apparent dissociation constants (K_D) for the complexes of monoclonal antibodies and AChE were calculated as described earlier (Brimijoin et al., 1983). Least-squares nonlinear regression analysis of binding data was carried out on a Hewlett-Packard Model 85 microcomputer with the aid of a program written by Peck and Barrett (1979).

RESULTS

Antibody production

Two mice were immunized with highly purified, partially heat-denatured rat brain AChE; two spleen cell–myeloma fusions were performed; nearly 150 primary hybridoma cultures were screened as described in Materials and Methods. We obtained seven cultures that secreted antibody against AChE. These were subcloned by two consecutive limiting dilutions to obtain a single, stable antibody-secreting cell line from each culture. The clonal cell lines were expanded as ascites tumors, and pure IgG was isolated from the ascites fluid (see Materials and Methods).

Sedimentation of AChE immune complexes

The effects of several monoclonal antibodies on the sedimentation velocity of rat brain AChE were examined to determine whether binding to multiple epitopes on each catalytic subunit was likely. To begin with the simplest possible sedimentation pattern, monomeric (G_1) AChE was prepared from crude extracts of "soluble" brain enzyme, where this form accounts for approximately half of the total AChE activity. When G_1 AChE was exposed for 1 h at 37°C to monoclonal antibody ZR5 in a concentration of 10^{-6} *M*, sedimentation analysis showed that all of the enzyme was converted to a single nonprecipitating complex (Fig. 1). The apparent sedimentation coefficient of the enzyme–antibody complex was about 7S, an increase of 3S over that of the free G_1 form. Similar patterns were obtained using other monoclonal antibodies. As will be discussed, this behavior is consistent with the assumption of single epitopes for each antibody on the catalytic subunits of AChE.

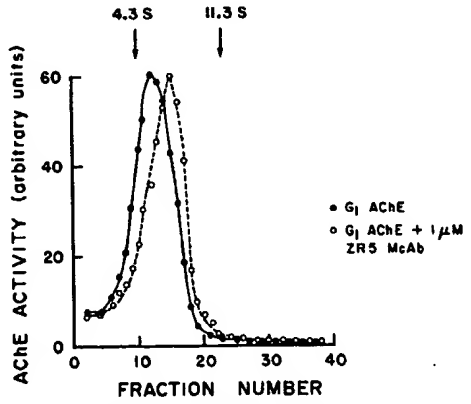

FIG. 1. Sedimentation velocity of AChE and immune complexes. Monomeric (G_1) AChE was first isolated on a 5–20% sucrose density gradient and was recentrifuged after 1 h incubation at 37°C in the presence or absence of ZR5 antibody, at a final concentration of 10^{-6} *M*. The arrows show the position of catalase (11.3S) and bovine serum albumin (4.3S) added as calibration standards.

Apparent affinity for rat brain AChE

To assess their affinity for antigen, various dilutions of immobilized antibody were incubated with freshly prepared crude extracts of "soluble" and "membrane-associated" rat brain AChE. After centrifugation the AChE activity was measured both in pellets and in supernatant fractions (see Materials and Methods). Typical binding curves for a relatively high-affinity antibody (ZR7) are shown in Fig. 2.

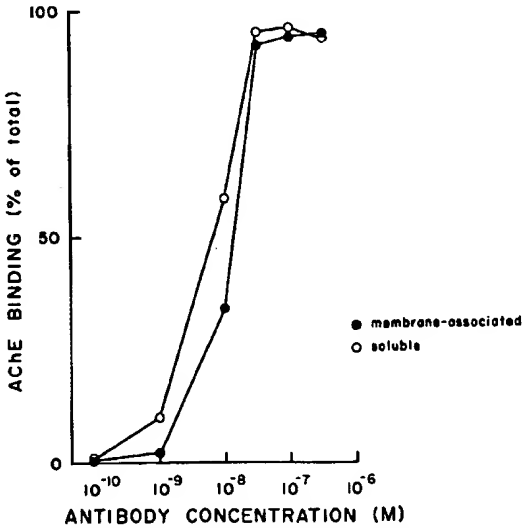

FIG. 2. Typical IgG dilution curves for binding of rat brain AChE by monoclonal antibodies. "Soluble" and "membrane-associated" AChE were crude extracts of brain homogenized, respectively, in the absence and presence of detergent (see Materials and Methods). Enzyme binding was determined by the solid-phase immunoadsorbance assay described in the text. The data are representative of the behavior of the highest affinity antibody (ZR7).

Apparent dissociation constants (K_D) were calculated from the binding data according to a previously described method (Brimijoin et al., 1983), using a computer-based nonlinear least-squares analysis. The calculation was not expected to give a true K_D because crude "soluble" and "membrane-associated" extracts of rat brain contain at least two molecular forms of AChE and because stoichiometric binding of IgG to the multimeric (i.e., 10S) form of AChE was unproven. Nevertheless, the derived constants are probably accurate within an order of magnitude and they do indicate the relative affinities of the different antibodies.

Comparison of the K_D values summarized in Table 1 shows that the range of affinities is about 100-fold. One of the monoclonal antibodies (ZR1) was of low affinity (K_D greater than 1000 n*M*). Another antibody (ZR7) was of high affinity (K_D, 10–30 n*M*). The remaining antibodies (ZR2–ZR6) formed a medium affinity group (K_D, 30–200 n*M*). It is worth noting that antibodies ZR2, ZR3, and ZR7 preferred the naturally soluble enzyme: all three bound soluble AChE with a K_D that was less than half the K_D for membrane-associated AChE. Antibody ZR5 showed a contrasting preference for the later type of enzyme.

Immunohistochemistry

Since their apparent affinities were moderately high, we tested whether the antibodies would stain AChE in frozen sections of formalin-fixed nervous tissue from the rat. Under optimal fixation conditions, specific immunofluorescence was detected in the striatum and the ventral horn of the spinal cord after treatment with pooled monoclonal antibody and fluorescein-conjugated second antibody (see Materials and Methods). The fluorescence was not intense when viewed directly but was dramatic when observed through a sensitive video system. Staining in the striatum was confined to neuron-rich regions, with the striations themselves appearing dark (Fig. 3A). The fluorescence was often brightest at or near cell surfaces (Fig. 3B). Much less fluorescence was noted in adjacent regions of brain (not shown) or in striatal sections treated with normal mouse serum in place of specific antibody (Fig. 3C and D). In the spinal cord, specific staining of large neurons and associated processes was clearly evident (Fig. 4).

TABLE 1. *Apparent dissociation constants (K_D) for complexes of monoclonal antibodies with rat brain "soluble" and "membrane-associated" AChE*

Antibody	Apparent K_D (n*M*)	
	"Soluble AChE"	"Membrane-associated AchE"
ZR1	1380 ± 236	1260 ± 160
ZR2	32 ± 5.0	83 ± 12
ZR3	62 ± 6.5	204 ± 39
ZR4	50 ± 5.3	68 ± 9.5
ZR5	56 ± 9.3	30 ± 6.7
ZR6	103 ± 10	89 ± 15
ZR7	14 ± 2.6	27 ± 6.5

Calculations were performed as previously described (Brimijoin et al., 1983) and were based on a one-site model (i.e., a single antibody binding site (epitope) per enzyme molecule).

Species cross-reactivity

Experiments were carried out to compare the cross-reactivity of the antibodies with brain AChE from other mammals and nonmammalian vertebrates. Each antibody was tested in a single high concentration (10^{-6} *M*) for its ability to bind AChE in fresh extracts of whole brain from mouse, human, rabbit, guinea pig, cow, cat, chicken, and frog. Tests were also made with commercially purified AChE from the electric eel. Although the actual volume of brain extract was slightly different in each case, the amount of AChE activity used was approximately the same (0.5 units).

As Fig. 5 shows, none of the antibodies could bind frog or electric eel AChE or had much affinity for the chicken brain enzyme. The cross-reactivities with mammalian AChEs, however, fell into five distinct patterns: essentially equivalent affinity for enzyme from each species including mouse (ZR1); almost exclusive preference for rat brain AChE (ZR2, ZR5, ZR6); a preference for enzyme of rat and guinea pig (ZR4); a preference for AChE of rat and cow (ZR7); limited cross-reactivity with AChE of rabbit, guinea pig, and cat (ZR3).

We tested whether any of the antibodies could distinguish among the brain AChEs from different inbred strains of mice, since any such abilities could be used to analyze the biochemical genetics of the enzyme. Crude brain extracts from 13 different inbred mouse strains with widely varying genetic backgrounds were used. Enzyme binding was determined by the routine solid-phase assay, using a single concentration of each monoclonal antibody (10^{-6} *M*). With fresh brain extracts, no consistent strain differences in binding of AChE were noted (Fig. 6). Storage of frozen brain extracts for 2 weeks, however, caused dramatic strain-specific losses in immunoreactivity that were not paralleled by losses of AChE activity. These effects were presumed to reflect differences in enzyme stability and were not pursued further.

Other properties

Isotype analysis showed that all the antibodies were IgG2b, with κ light chains. According to Kronvall et al. (1970), Protein A has a high affinity for this class of immunoglobulins, and Protein A-bearing Pansorbin should bind them well. An experiment was therefore done to determine whether rabbit antimouse IgG was required as a "linking antibody" to precipitate AChE in the solid-phase binding assay. The results showed less than optimal immunoprecipitation without the linking antibody, except when ZR4 was used (Table 2).

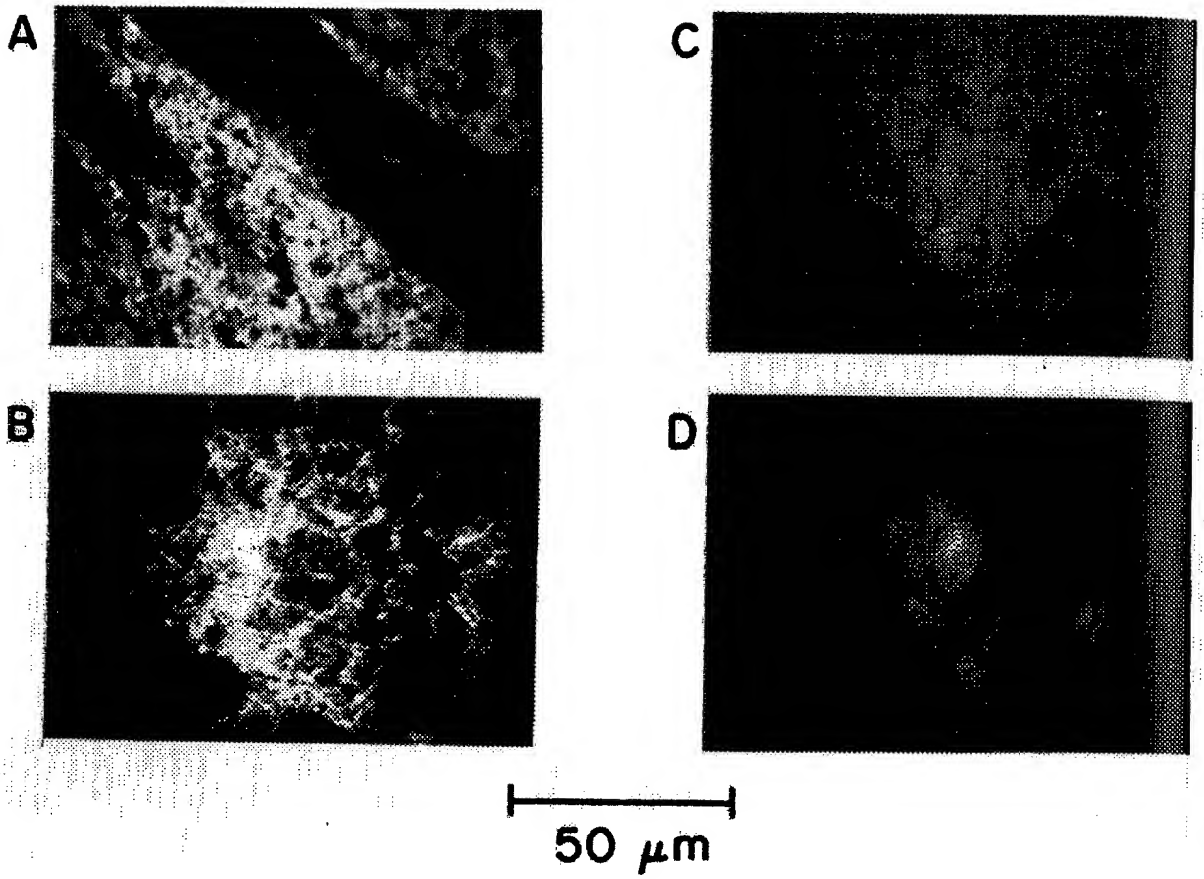

FIG. 3. Indirect immunofluorescence staining of rat striatum. Cryostat sections (12 μm) were cut from formalin-fixed rat brain and treated with pooled monoclonal antibody at 1:30 dilution, followed by fluorescein-conjugated goat anti-mouse IgG. Fluorescence was observed on a Zeiss Axiomat microscope (×16 objective) with the aid of a silicon-target-intensified video camera and video monitor. Photographs were made directly from the monitor. **A:** Striatum, showing general staining of cell bodies and lack of staining in the interpenetrating striations. **B:** Brightly staining region of the caudate nucleus, with numerous small cells outlined by fluorescent antibody. **C and D:** Background fluorescence in control sections of striatum stained with normal mouse serum in place of specific antibody.

Salt sensitivity was tested by performing the solid-phase binding assay with each antibody, at concentrations ranging from 10^{-9} to 10^{-6} *M*, in the presence or absence of 1 *M* NaCl. The results (summarized in Table 2) showed that the affinity of ZR1 was slightly reduced in the presence of salt, while the other antibodies were essentially unaffected. Detergent sensitivity, tested similarly by investigating binding of "soluble AChE" in the presence or absence of 1% Triton X-100, was equally low (data not shown).

It was also investigated whether any of the antibodies could inhibit rat brain AChE. The enzyme was incubated for 1 h at 37°C in the presence or absence of antibody (10^{-6} *M*) and the AChE activity of the mixtures was measured spectrophotometrically. None of the antibodies measureably affected the activity except ZR2, which caused a trivial inhibition (Table 2).

DISCUSSION

There have been previous but unsuccessful attempts to produce monoclonal antibodies to rat brain AChE. According to Marsh et al. (1984), a chief source of difficulty could be extensive immunochemical similarities among rodent AChEs. However, Mintz and Brimijoin (1985) identified two monoclonal antibodies to rabbit brain AChE that cross-reacted with the enzyme of rat but not mouse. Our current results confirm the inference that there are significant structural differences among the brain AChEs of different rodents. Two factors that may have aided antibody production in the present case were (a) immunization with highly purified, essentially homogeneous enzyme; (b) partial heat inactivation of the antigen which, after denaturation, could have seemed more "foreign" to the murine immune system.

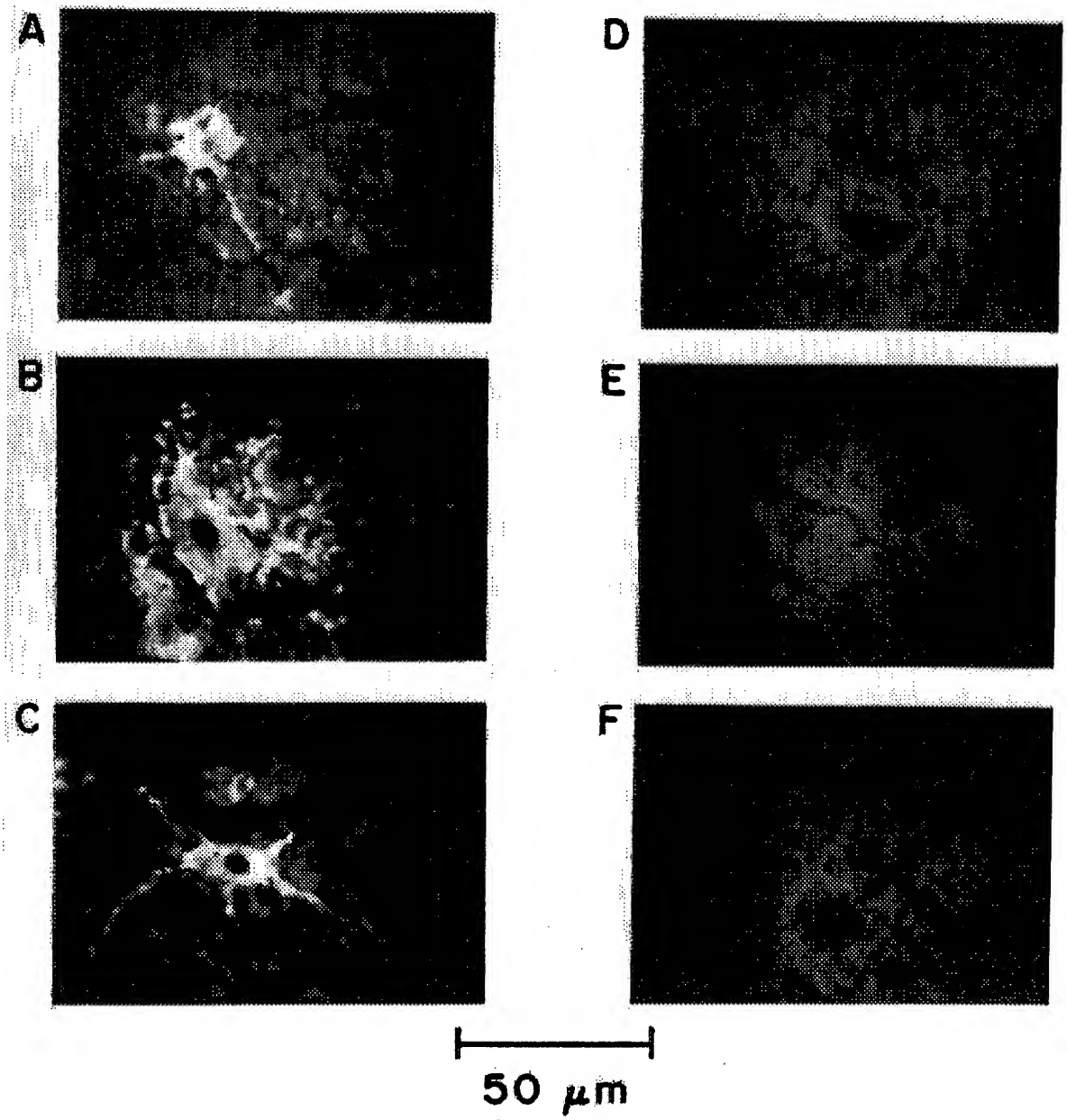

FIG. 4. Indirect immunofluorescence staining of rat spinal cord. Cross sections of spinal cord were treated and photographed as described in the legend to Fig. 3. **A–C:** Specific fluorescence induced by pooled monoclonal antibody. **D–F:** Background fluorescence induced by normal mouse serum.

By sedimentation analysis, we characterized the immune complexes formed between the monoclonal antibodies and the G_1 AChE of rat brain. Only one type of complex was produced, with a sedimentation coefficient of about 7S, even though the antibodies were added in large excess (concentrations about 20 times the apparent K_D). This behavior is consistent with the effects of monoclonal antibodies against human AChE when tested with different molecular forms of this enzyme (Fambrough et al., 1982; Brimijoin et al., 1983; Marsh et al., 1984). An extensive study of immune complexes with AChE forms from human red blood cells (Brimijoin and Mintz, 1985) demonstrated that similar conditions led to the binding of one IgG molecule per catalytic subunit, increasing the sedimentation coefficient by 5S or less. It is therefore probable that 1:1 complexes of enzyme and immunoglobulin were generated in the present case.

The affinity of the newly isolated monoclonal an-

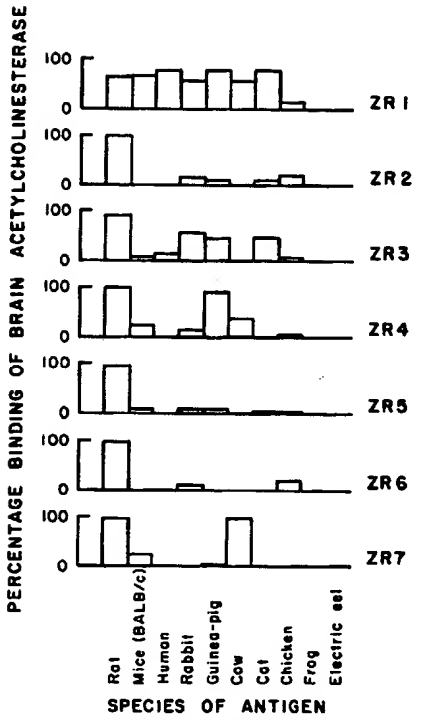

FIG. 5. Cross-reactivity of monoclonal antibodies with brain AChE from various animal species. Each binding assay was performed with a variable amount of crude detergent extract of brain tissue with approximately 0.5 units of AChE activity. Antibody concentration was 10^{-6} *M* in all cases.

tibodies is important in determining their utility as tools for immunohistochemistry, immunoassay, or immunopurification of rodent AChE. Monoclonal antibodies to other AChEs have bound the rat enzyme only weakly (Mintz and Brimijoin, 1985). By comparison, the present antibodies bound tightly enough to produce clear-cut immunofluorescence staining of central nervous tissue. The apparent dissociation constants, ranging down to about 20 n*M*, are small enough to make additional applications of the antibodies feasible. Although the dissociation constants by themselves give no information about the nature of the binding sites recognized, they do suggest that each isolated antibody is a distinct molecule with unique properties.

The pattern of antibody cross-reactivities with AChEs from different animal species is in some respects as important as absolute affinity because it may reveal similarities and differences among immunogenic sites (epitopes) on the enzymes. For example, the broad cross-reactivity of ZR1 with the brain AChEs of various mammals indicates that at least one AChE epitope is shared by this group of animals, though lacking in the other vertebrates tested. It is interesting that ZR1 bound mouse AChE as avidly as rat AChE, implying a breakdown in immune tolerance. The fact that none of our monoclonals react with electric eel AChE is in agreement with previous reports that polyclonal antibodies to the *Electrophorus* enzyme did not cross-react with mammalian AChE (Williams, 1969) and vice versa (Greenberg et al., 1977).

Taken together, our findings indicate that each of the seven isolated AChE antibodies is a unique molecule. The five distinct patterns of species cross-reactivity suggest that at least five different epitopes are recognized. At present we cannot exclude the possibility that the antibodies with similar cross-reactivities (ZR2, ZR5, and ZR6) are directed against the same epitopes. However, these antibodies differ in their relative affinities for the brain AChEs extracted with and without detergent. It therefore seems likely that they actually do bind to different portions of the AChE surface.

The ability of some antibodies to distinguish between the "soluble" and "membrane-associated" AChE of rat brain deserves further comment. The preference of three antibodies for soluble AChE is surprising since the immunizations were performed with "membrane-associated" enzyme. We doubt that the reduced affinity for membrane-associated AChE arises simply from the presence of Triton X-100 in the buffers, because Triton had no effect on the apparent affinity for soluble AChE. Conceivably the antibodies in question bind to sites that, on membrane-associated AChE only, are partially blocked by Triton. However, the present results

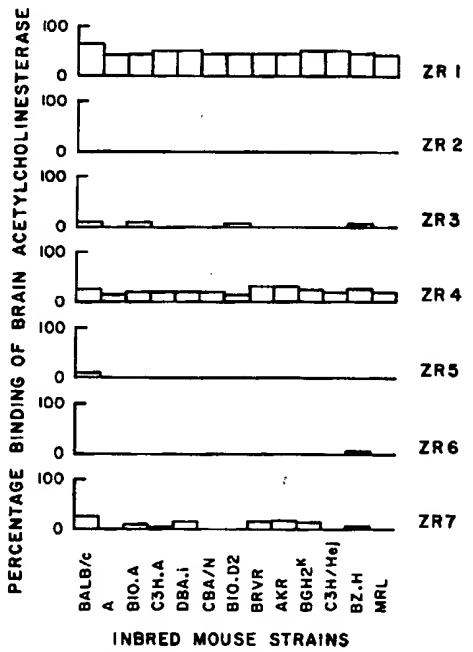

FIG. 6. Cross-reactivity of antirat brain AChE monoclonal antibodies with brain AChE from various inbred mouse strains. Conditions of the binding assays were as described in the legend to Fig. 5.

TABLE 2. *Summary of properties of monoclonal antibodies to rat brain AChE*

Antibody	IgG isotype	Binding to Pansorbin[a]	Binding to RAM-Pansorbin[b]	Affinity	Salt-sensitivity[c]	Preference for soluble AChE	Inhibition of AChE[d]
ZR1	2b	+ +	+ + (~60%)	+	+ (<10%)	–	..
ZR2	2b	+ +	+ + + (>95%)	+ +	–	+ +	+ (<10%)
ZR3	2b	+ (<20%)	+ + +	+ +	–	+ +	–
ZR4	2b	+ + + (>80%)	+ + +	+ +	–	–	–
ZR5	2b	+ +	+ + +	+ +	–	–	–
ZR6	2b	+ +	+ + +	+ +	–	–	–
ZR7	2b	+ +	+ + +	+ + +	–	+ +	–

Results are indicated here in semiquantitative form: –, absent or indetectable; +, low; + +, moderate; + + +, high.
[a] The solid-phase binding assay was performed directly with Pansorbin. Monoclonal antibody concentration was 10^{-6} *M*.
[b] Same as above except Pansorbin was treated with rabbit anti-mouse immunoglobulin.
[c] The solid-phase binding assay was performed in the presence and absence of 1 *M* NaCl.
[d] Approximately 0.5 unit of AChE was incubated for 1 h at 37°C in the presence and absence of monoclonal antibodies (10^{-6} *M*).

should be evaluated in the light of earlier suggestions of immunological differences between the soluble and particulate fractions of AChE in rat brain (Zanetta et al., 1981) and human caudate nucleus (Sørensen et al., 1982). Although these reports have been challenged (Marsh et al., 1984), the issue will remain open until the immunochemistry of AChE forms has been thoroughly analyzed. The anti-rat AChE monoclonal antibodies may be useful for this purpose.

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REFERENCES

Adamson E. D. (1977) Acetylcholinesterase in mouse brain, erythrocytes and muscle. *J. Neurochem.* 28, 605–615.

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 92, 248–254.

Brimijoin S. and Mintz K. P. (1985) Human acetylcholinesterase. Immunochemical studies with monoclonal antibodies. *Biochim. Biophys. Acta* 828, 290–297.

Brimijoin S., Mintz K. P., and Alley M. C. (1983) Production and characterization of separate monoclonal antibodies to human acetylcholinesterase and butyrylcholinesterase. *Mol. Pharmacol.* 24, 513–520.

Bruck C., Portetelle D., Glineur C., and Bollen A. (1982) One step purification of mouse monoclonal antibodies from ascitic fluid by DEAE Affi-Gel Blue chromatography. *J. Immunol. Methods* 52, 313–319.

Coons A. H. (1958) Fluorescent antibody methods. In *General Cytochemical Methods* (Danielli J. F., ed), pp. 399–422. Academic Press, New York.

Ellman G. L., Courtney K. D., Andres V. Jr., and Featherstone R. M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7, 88–95.

Fambrough D. M., Engel A. G., and Rosenberry T. L. (1982) Acetylcholinesterase of human erythrocytes and neuromuscular junctions: homologies revealed by monoclonal antibodies. *Proc. Natl. Acad. Sci. USA* 79, 1078–1082.

Fazekas de St. Groth S. and Scheidegger D. (1980) Production of monoclonal antibodies: strategy and tactics. *J. Immunol. Methods* 35, 1–21.

Greenberg A. J., Parker K. K., and Trevor A. J. (1977) Immunochemical studies of mammalian brain acetylcholinesterase. *J. Neurochem.* 29, 911–917.

Johnson C. D. and Russell R. L. (1975) A rapid simple radiometric assay for cholinesterase, suitable for multiple determinations. *Anal. Biochem.* 64, 229–238.

Köhler G. and Milstein C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256, 495–497.

Kronvall G., Grey H. M., and Williams R. C. Jr. (1970) Protein A reactivity with mouse immunoglobulins. *J. Immunol.* 105, 1116–1123.

Marsh D. M., Grassi J., Vigny M., and Massoulié J. (1984) An immunological study of rat acetylcholinesterase: comparison with acetylcholinesterases from other vertebrates. *J. Neurochem.* 43, 204–213.

Mintz K. P. and Brimijoin S. (1985) Monoclonal antibodies to rabbit brain acetylcholinesterase: selective enzyme inhibition, differential affinity for enzyme forms, and cross-reactivity with other mammalian cholinesterases. *J. Neurochem.* 45, 284–292.

Peck C. C. and Barrett B. B. (1979) Nonlinear least squares regression programs for microcomputers. *J. Pharmacokinet. Biopharm.* 5, 537–541.

Rakonczay Z., Mallol J., Schenk H., Vincendon G., and Zanetta J.-P. (1981*a*) Purification and properties of the membrane-bound acetylcholinesterase from adult rat brain. *Biochim. Biophys. Acta* 657, 243–256.

Rakonczay Z., Vincendon G., and Zanetta J.-P. (1981*b*) Heterogeneity of rat brain acetylcholinesterase: a study by gel filtration and gradient centrifugation. *J. Neurochem.* 37, 662–669.

Schwabe C. (1973) A fluorescent assay for proteolytic enzymes. *Anal. Biochem.* 53, 484–490.

Sørensen K., Gentinetta R., and Brodbeck V. (1982) An amphiphile-dependent form of human brain caudate nucleus acetylcholinesterase: purification and properties. *J. Neurochem.* 39, 1050–1060.

Williams R. M. (1969) Antibodies to acetylcholinesterase. *Proc. Natl. Acad. Sci. USA* 62, 1175–1180.

Zanetta J.-P., Rakonczay Z., Reeber A., Kasa P., and Vincendon G. (1981) Antibodies against the membrane-bound acetylcholinesterase from adult rat brain. *FEBS Lett.* 129, 293–296.